REMARKS

Applicants have amended claims 1, 18, 22 and 24 to refer to amplification with PCR using a duplex reaction using two different sets of target and control specific primers. Support for the amendment can be found throughout the specification, for example on page 16, lines 17-20, and page 19, lines 6-10. Applicants have further amended these claims to make explicit that which was implicit, namely, that the primer extension step of the methods require sequential primer extension reactions to result in sequential analysis of control and the target sequences (see, e.g., page 20, lines 32-33), and sequential dispensation of nucleotides in a predetermined order in such a way that the amplicons that are present in one reaction mixture will be analyzed separately as indicated as indicated on page 19, lines 18-30. To expedite prosecution, Applicants have cancelled the previously withdrawn claims 32-34, 37, without prejudice.

Applicants now turn to the specific rejections.

The Examiner rejected claims 1-9, 15-26, 35-36, 38 and 39 under 35 U.S.C. §103(a) as allegedly obvious over U.S. Patent Application Publication No. 20030054386 to Antonarakis et al ("Antonarakis") in view of U.S. Patent No. 5,705,365 to Ryder et al. ("Ryder"). Specifically, the Examiner alleges that Antonarakis teaches co-amplification of a target and control to quantitate the amount of nucleic acids. The Examiner further alleges that although Antonarakis does not teach stopping the reaction at exponential phase but that Ryder teaches this element.

Applicants respectfully disagree and submit that the rejection should be withdrawn for the following reasons.

Applicants respectfully submit that the method described in Antonarakis significantly differs from the presently claimed method.

In addition, Antonarakis uses a single pair of primers to amplify both the first and the second sequences (see, e.g., par [0013]). Whereas, the presently claimed method amplifies the target and control sequences using two different target and control specific primer pairs. Antonarakis also performs the primer extension simultaneously for both the paralogous sequences in the sample (see, e.g., par.[0084]). Whereas the presently claimed method requires sequential primer extension reaction. Given the parameters of the method of Antonarakis, their method cannot be used unless the control and target sequences share a significant sequence homology, whereas the presently claimed method also allows comparison between completely different sequences.

In other words, Antonarakis produces **one single pyrogram,** which **represents** the **combination of the sequences** present in the analyzed mixture. Thus, each peak represents a combination of the two sequences except for those locations where the sequences of the two differ. In contrast, the presently claimed method produces essentially **two sequential reaction products, such as pyrograms** from the two different primers **representing each one of the samples separately**. Each peak thus represents the accurate amount from one single sequence. This allows one to compare the average peak sizes of the two different reaction products, such as pyrograms to accurately quantify the amount of target compared to the control. This is only possible by **sequential analysis** of the target and the control as explained, for example at page 20, last paragraph and page 21, first paragraph.

Thus, the sequential analysis of control and target sequences is not taught by Antonarakis.

Applicants respectfully submit that the amendments to the claims have made the differences between Antonarakis and the present invention based on sequential analysis of the primer extension reactions explicit.

Ryder does not cure this deficiency. Ryder does not teach using sequential analysis of the primer extension products in a duplex sample. Accordingly, Ryder does not provide a teaching for this element in the claims.

Applicants therefore respectfully submit that the cited references do not teach all the elements in the present method.

Moreover, Applicants submit that a skilled artisan neither would have been motivated to combine the references nor have had a reasonable expectation of success in combining the references for the following reasons.

The Examiner alleged that Ryder teaches an amplification method wherein amplification remains in exponential phase and that thus one skilled in the art would have been motivated to combine Ryder with Antonarakis.

Applicants respectfully disagree.

Applicants further submit that Antonarakis specifically indicated that to generate suitable quantities of nucleic acids to be quantified using their method, 35 cycles of PCR must be performed (par. [0123]). After 35 cycles, PCR amplifications are typically in plateau phase which prevents appropriate quantification of the target nucleic acids. Thus, a skilled artisan

would not have been motivated to consider a quantification method in connection with the method of Antonarakis, which needs so. In other words, one would have dismissed a method wherein one stops the reaction mixture before the plateau because such method would noy have resulted in sufficient amount of material for the subsequent analysis by the methods of Antonarakis

Ryder also specifically teaches that PCR suffers from the problem of not remaining in exponential phase and suggests that **instead of PCR one should use a different method**. Thus, Ryder teaches against using PCR in quantitative methods in general thus teaches away from combining the references in a PCR based method to which the claims are now directed to.

Moreover, use of two different extension primers as required by claim 4 is not taught in either references.

In view of the above, Applicants respectfully submit that the rejection of claims 1-9, 15-26, 35-36, 38 and 39 under 35 U.S.C. §103(a) over Antonarakis in view of Ryder, should be withdrawn.

The Examiner rejected claims 10-14 under 35 U.S.C. §103(a) as allegedly obvious over Antonarakis in view of Ryder and further in view of Pourmand et al. (Nucleic Acids Research, 30(7):e31, 2002)("Pourmand").

Applicants respectfully disagree and submit that the rejection should be withdrawn for the following reasons.

As described, *supra*, the combination of Antonarakis and Ryder does not teach all the elements in the present claims, namely, that in a duplicate reaction, one performs **primer extension reaction sequentially**.

Pourmand does not cure this deficiency. Although Pourmand describes use of different extension primers, it also teaches that the **product is one pyrogram with a "unique pattern"** (see, e.g., the abstract). The "raw data" is in a single pyrogram that requires correction based upon the nucleic acid dispensation order and predicted pyrogram patterns (see, e.g., Figure 2). There is nothing in Pourmand that teaches or suggests that the different sequences can or should be analyzed sequentially. Moreover, Pourmand does not provide any description how one would use the multiplex pyrosequencing for comparative quantification of nucleic acid sequences.

Therefore, Applicants respectfully submit that the rejection of claims 10-14 under 35 U.S.C. §103(a) over Antonarakis in view of Ryder, and further in view of Pourmand should be withdrawn.

The Examiner rejected claims 1-9, 15-26, 35-36, 38 and 39 under 35 U.S.C. §103(a) as allegedly obvious over Antonarakis in view of U.S. Patent No. 5,858,658 to Haemmerle et al. ("Haemmerle").

Applicants respectfully disagree and submit that the rejection should be withdrawn for the following reasons.

As described, *supra*, the combination of Antonarakis does not teach all the elements of the present claims, namely the sequential analysis of the primer extension reaction products. Moreover, the combination does not teach using different control and target sequence-specific primers in a duplex PCR.

Haemmerle does not cure this deficiency. Haemmerle specifically requires that the standard and the target "should be capable of being amplified by aid of **the same primers**" (col. 3, lines 1-4, emphasis added). Haemmerle also specifies, that in analyzing the quantity of the standard and the target after the amplification "in most cases, however, a step **must be provided** in which the amplified standard nucleic acid is separated from the amplified genomic DNA..." (col. 3, lines 28-38, emphasis added). Thus, the analysis of the primer extension reaction is not performed in the same reaction mixture as required by the claims.

Accordingly, the combination of Antonarakis and Haemmerle fails to teach the element of using two different primers for a duplex PCR in a quantitative analysis, and a sequential analysis in the same reaction mixture of the resulting primer extension products.

Therefore, Applicants respectfully submit that the rejection of claims 1-9, 15-26, 35-36, 38 and 39 under 35 U.S.C. §103(a) over Antonarakis in view of Haemmerle, should be withdrawn.

The Examiner rejected claims 10-14 under 35 U.S.C. §103(a) as allegedly obvious over Antonarakis in view of Haemmerle and further in view of Pourmand.

As described, *supra*, the combination of Antonarakis and Haemmerle does not teach all the elements in the present claims, namely, that one amplifies the target and control sequences with respective specific primers in a duplicate reaction, and that one analyses the primer extension reactions in a same reaction mixture **sequentially**.

Pourmand does not cure this deficiency. As described, *supra*, Pourmand does not teach or suggest or let a skilled artisan to expect success in sequential analysis of the target and control sequences in primer extension reaction.

Accordingly, Applicants respectfully submit that the rejection of claims 10-14 under 35 U.S.C. §103(a) over Antonarakis in view of Haemmerle, and further in view of Pourmand should be withdrawn.

As a further explanation, Applicants attach herewith a drawing illustrating the differences between the sequential primer analysis method of the present invention and the analysis methods of the prior art. The drawing is attached as Exhibit A. Exhibit A shows an excerpt of Figure 3 of the present application on the left, wherein peaks from the first primer are directly compared to peaks from the second primer (indicated below). In contrast, the Figure 3 of Pourmand is excerpted to the right, and it shows that in order to discern the sequence of the multiplex primed samples on the bottom of section C, one must revert back to single pyrograms (B) or predicted combined sequences (C, top), to determine the sequence. There is no indication or guidance as to how one would determine the relative quantities of the multiple primed template, where the peaks are detected in random order from each of the templates.

In view of the above, Applicants respectfully submit that the claims are in condition for allowance. Early and favorable consideration is sincerely solicited.

If any fee deficiencies are associated with this submission, the Commissioner is authorized to debit such deficiencies to the Nixon Peabody Deposit Account No. 50-0850. Any overpayments should be credited to the same Deposit Account.

Customer No. 50828

Respectfully submitted,

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